56. The method as claimed in claim 55, wherein the oligomers are labeled with ^{32}P.

57. The method as claimed in claim 53, wherein the oligonucleotides have a length of from 8 to 20 nucleotides.

58. The method as claimed in claim 53, wherein the oligonucleotides are attached to the surface as an array of a parallel stripes, and at least two polynucleotides are analyzed simultaneously by applying the polynucleotides to the array in the form of separate stripes orthogonal to the oligonucleotide stripes.

59. The method as claimed in claim 53 wherein hybridization is effected in the presence of tetramethylammonium chloride at a concentration of 1M to 5M.--

REMARKS

Favorable reconsideration is respectfully requested.

The claims are 40 to 59.

The above amendment presents a new set of claims to replace those previously on file.

Basis for the new claims is as follows:

New claim 40 corresponds to old claim 17 with added limitations.

New claims 41, 42 and 43 correspond to old claim 18, but each contains an added limitation.

Claim 40 is an apparatus claim, and claim 53 is a corresponding method claim, directed to "analyzing an undetermined sequence or undetermined sequence variant" (page 5, line 19 to page 6, line 14; page 27, line 1 to page 29, line 26). The claim contains the phrase "under conditions which allow oligonucleotides which have complements in the polynucleotide to be distinguished from those which do not". This phrase is based on the specification at page 7, lines 30 to 33.

Claim 41 is an apparatus claim directed to the diagnostic mode, and contains the phrase "each oligonucleotide being attached to the surface of the support through a terminal nucleotide,". This phrase finds support in the specification at page 12, lines 23 to 25.

Claim 42 is an apparatus claim directed to the diagnostic mode, and contains the phrase "each oligonucleotide having been synthesized *in situ* and being attached to the surface of the support through a covalent linkage". This phrase finds support at page 12, lines 5 to 25.

Claim 43 is an apparatus claim directed to the diagnostic mode, and recites that the oligonucleotides have lengths chosen to discriminate between matches and mismatches. This recitation finds support at page 2, lines 21 to 25 and in Examples 3 and 4.

New claims 44, 45, 46, 47 and 48 correspond to old claims 19, 20, 38, 22 and 23.

New claims 49, 50, 51 and 52 correspond to old claims 28, 29, 30 and 31.

New claim 53 corresponds to old claim 26.

New claims 54, 55, 56, 57, 58 and 59 correspond to old claims 27, 33, 34, 35, 36 and 37.

The significance of the new claims will become further apparent from the remarks below.

The counterpart European patent application has been granted as European Patent EP 0 373 203 B. This European patent has been opposed by opponents who have cited a total of 60 references. Of these, just five had been previously brought to the attention of the U.S. Patent Office. Thus, enclosed is an Information Disclosure Statement submitting the remaining 55.

Of these 55 new references, one is believed to be of particular interest (Category A), namely EPA 235,726 of Molecular Diagnostics/Dafta Gupta. A detailed commentary on this reference is attached to this response. The other 54 references are either completely irrelevant (Category C) or else merely cumulative of art already before the Examiner (Category B).

This additional art came to applicant's attention around June 1995. In order to save the USPTO Examiner unnecessary work, applicant reviewed the art before submitting it. But that process has taken several months which is why the Information Disclosure Statement is only being submitted now.

The above amendment takes into account (distinguishes over) the newly cited references.

With regard to the requirement for a new and more descriptive title, such is submitted herewith.

Turning to the remaining objections/rejections:

35 USC 112, First Paragraph.

The Official Action objects that there is no written basis for old claim 19 (new claim 44). It notes that the closest disclosure is found at page 17, line 17 to page 18, line 19, but finds no written basis for separate steps b) to d) wherein a first, second and third set of cell locations are each separately coupled with a nucleotide with continuation of this sequence of coupling steps until the desired array is generated.

In reply, please refer to the present specification and Examples 1, 2 and 3. In each of these examples, an array of oligonucleotides was built up by segregating a support material into discrete cell locations, coupling a nucleotide to one set of cell locations; then to another set; then to a third set; and continuing the sequence of coupling steps until the desired array has been generated. Thus in Example 3, lengths of silicone rubber tubing were used to define discrete cell locations on a support material (a microscope slide). Manifestly, the arrays shown in Table 1 on page 30 were made by the method defined in new claim 44.

Accordingly, there is adequate support for claim 44 and dependent claims 45 to 48 in the light of the above comments.

The above comments are also responsive to the rejection of claims 19, 20, 22, 23 and 38 under 35 USC 112, first paragraph.

35 USC 112, Second Paragraph.

Claims 17, 18, 25 to 37 and 39 have been rejected under 35 USC 112, second paragraph, as indefinite in the term "defined sequence" or "defined sequences".

In reply, in the new claims submitted herewith, claims 40 and 53 refer to "oligonucleotides with defined sequences", and claims 41, 42 and 43 refer to "oligonucleotides with known sequence". The Official Action states that no definition of the word "defined" is provided. Applicant replies that he is using "defined" in its ordinary English dictionary sense, and that one of ordinary skill in the art could easily tell whether a "defined" sequence is involved, particularly in the light of the specification.

The Official Action further states that the phrase "oligonucleotides of defined sequence" might be interpreted to mean that the sequences are defined by their source of procurement. That this statement is not right is a simple matter of English grammar. An oligonucleotide may be defined, e.g., by its sequence or by its source of procurement. But in the phrase

used in applicant's claims, it is the sequence, not the oligonucleotide, which is "defined" or "known". In the phrase "oligonucleotides of defined sequence", a sequence is merely a list of nucleotides. As applied to such a list of nucleotides, the word "defined" can have only one meaning. Favorable reconsideration is therefore respectfully requested.

Dates of Claims.

The Official Action has assigned priority dates to particular claims. The term "priority date" is misdescriptive, because the subject matter of these claims may possibly have an earlier U.S. date of conception than the date of filing of any patent application. With that proviso:

Applicant agrees that new claims 40, 41, 42, 43, 53, 54 and 55 to 59 (corresponding to old claims 17, 18, 26, 27, 32 to 36 and 39) are fairly based on the disclosure of the priority British patent application filed May 3, 1988.

The Official Action has said that old claims 25 and 28 to 31 are entitled to a priority date of only May 2, 1989. However, claim 25 has been deleted without prejudice, and claims 28 to 31 have been replaced by new claims 49 to 52, dependent on claims 41, 42 and 43. These new claims 49 to 52 are fairly based on the priority British patent application filed May 3, 1988, because the features thereof are clearly disclosed in the specification of that British application.

The Official Action asserts that new claims 44 to 48 (old claims 19, 20, 22, 23 and 38) are entitled to a priority date no earlier than the date of filing of CIP application 07/695,682, filed May 3, 1991. Applicant respectfully disagrees, and submits that all these claims are fairly based on the specification of the British priority application filed May 3, 1988. Attention is directed particularly to Section 4.3 on pages 7 to 9 and Sections 5.2 and 5.3 on pages 11 and 12. Section 4.3 describes segregating a support into discrete cell locations. The reader is sufficiently taught in Section 5.3 how to provide a single oligonucleotide tethered to a support. If he can make one oligonucleotide, the reader can make several, tethered at spaced locations as indicated in Section 5.2. If he can make several immobilized oligonucleotides on a support, he can make an array.

As discussed above, Examples 1, 2 and 3 of Serial No. 07/573,317 provide additional support, which means that new claims 44 to 48 are, in any event, entitled to a priority date of May 2, 1989 or earlier.

Applicant further points out that there is support for new claim 44 in the specification of the original filing of May 3, 1988; e.g., section 5.2, "Laying Down the Matrix". In this section, it is envisaged that "the matrix (shorthand for an array of oligonucleotides) will be synthesized in the cells of an array by laying down the precursors for the four bases in a predetermined pattern...". Taken with the rest of this section,

a person skilled in the art could only interpret the intention to be what is more precisely described in claim 44: namely, to lay down precursors, in specified positions, sequentially to create different oligonucleotides in different cells. Section 4.3 teaches how to make an array of all oligonucleotides of a chosen length, so that each oligonucleotide occurs once and only once in the array. The sequential process described in 44 b to d follows from the well-known method for the chemical synthesis of oligonucleotides, which was assumed to be obvious to a reader skilled in the art. The novelty embodied in section 5.2 and claim 44 is the notion of applying conventional synthesis in a predetermined pattern to create an array.

Claims 25 and 39 have been rejected under 35 USC 102 as anticipated by Mundy. In reply, claims 25 and 39 have been deleted, and there are no counterparts of those claims in the new set now presented. Thus, this rejection is moot. In fact, Mundy concerns a development of the standard dot-blot assay format, in which a target is immobilized and is addressed by labeled probes in solution. This is very different from and unsuggestive of applicant's reverse dot-blot assay format.

Claims 25 to 28, 32 and 39 have been rejected under 35 USC 102(b) as anticipated by Saiki. In reply, Saiki describes a standard dot-blot assay format in which a target, a PCR amplified fragment of a β-globin gene, is immobilized and addressed by radiolabelled oligonucleotide probes (19a, 19s, 19c) in solution.

The Saiki disclosure is remote from and unsuggestive of the reverse dot-blot assay format on which applicant's invention is based.

Claims 25, 32 and 39 have been deleted, and no counterparts appear in the new set of claims now presented.

Old claim 26 has been replaced by new claim 53 which is directed to a method of analyzing "an undetermined sequence or undetermined sequence variant of a polynucleotide". The claim defines a method which involves applying that polynucleotide, having an undetermined sequence or an undetermined sequence variant, to an array of oligonucleotide of defined sequence immobilized on a support. This is in contrast to Saiki, who uses oligonucleotide probes of precisely known sequence in solution.

Old claim 27 has been replaced by new claim 54 which stands or falls with claim 53 on which it depends.

Old claim 28 corresponds to new claim 49 which is dependent upon any one of claims 41, 42 and 43. Claim 41 recites that the oligonucleotides of each cell are attached to the surface of the support through a terminal nucleotide. Claim 42 recites that the oligonucleotides of each cell of the array have been synthesized *in situ* and are attached to the surface of the support through a covalent linkage.

Claim 43 recites that each oligonucleotide of the array has a length of 8 - 20 nucleotides. These features serve to

render claims 41, 42 and 43, and also claim 49 depending on them, novel over and unobvious from Saiki.

Claims 25, 26, 27, 30, 33, 34 and 39 have been rejected under 35 USC 102(b) as anticipated by Brigati. All of the rejected claims have been deleted, and some have been replaced in the new set of claims presented by the above amendment. The concordance is as follows:

Old claim	25, 26, 27, 30, 33, 34, 39
New claim	--, 53, 54, 51, 55, 56, --

Brigati has been previously discussed, e.g., in the response of March 3, 1994 at page 6. The target for analysis is an infected cell culture or tissue section immobilized on a microscope slide. The probes are biotin-labeled viral DNA. The probes in solution are applied to the immobilized target and used to detect viral DNA and RNA sequences in the target.

This concept is remote from the concept underlying applicant's invention, which is a reverse dot-blot assay format in which a target in solution is applied to an array of immobilized oligonucleotide probes.

Claims 25 and 39 have been deleted without prejudice and have no counterparts in the new set of claims now on file. Thus, the rejection of these claims as anticipated by Brigati is moot.

New claim 53 is novel over and unobvious from Brigati in several respects:

a) Claim 53 is directed to a method of analyzing an undetermined sequence or undetermined sequence variant of a polynucleotide. In contrast, Brigati is merely concerned with sequence detection.

b) The method of claim 53 involves the use of a support to the surface of which are attached oligonucleotides of defined sequence, said oligonucleotides being attached to different locations on the surface of the support - in other words an array of different oligonucleotides of defined sequence. "Oligonucleotide" is a term of art, which is generally understood not to apply to cellular or genomic DNA or RNA. Brigati has one target immobilized on the surface of a microscope slide; the target is not an oligonucleotide and its DNA or RNA sequence is not defined. Brigati contains no disclosure of an array with different entities attached to different locations on a support.

c) The method of claim 53 involves applying the polynucleotide, having an undetermined sequence or undetermined sequence of variant to be analyzed, under hybridization conditions to the surface. In contrast, the method of Brigati is not directed to determining anything about the labeled viral DNA he uses as probes.

New claims 54, 55 and 56 are dependent upon claim 53 and are also patentable with claim 53.

New claim 51 is dependent upon claims 41, 42 or 43. Claim 41 is directed to an array of oligonucleotides with the

oligonucleotide of each cell being attached to the surface of a support through a terminal nucleotide. Claim 42 is directed to an array in which the oligonucleotides of each cell are synthesized *in situ* and are attached to the surface of a support through a covalent linkage.

Claim 43 is directed to an array of oligonucleotides each having a length of 8 to 20 nucleotides. By these features, claims 41, 42 and 43, and also 51 with them, are also novel over and unobvious from Brigati.

The Official Action rejects claims 17, 18, 25, 26, 28, 29, 31, 32, 35, 37 and 39 as unpatentable under 35 USC 103 over Hafeman in view of Macevicz or Wood. All of the rejected claims have been deleted without prejudice, and some are replaced in the set presently on file. The concordance is as follows:

Old claim 17, 18, 25, 26, 28, 29, 31, 32, 35, 37, 39

New claim 40, 41, 42, 43, --, 53, 44, 50, 52, --, 57, 59, --.

Hafeman discloses apparatus and methods for detecting analytes employing semiconductor capacitance as the signal modulated by the analyte. As disclosed at column 10, lines 26 to 34, the method may involve reaction between two members of a specific binding pair, which may (column 13, lines 50 to 66) be two complementary nucleic acid chains. A semiconductor surface may carry an array of probes, each consisting of one member of the specific binding pair, at spaced locations or pixels (column 2, lines 26 to 31).

Hafeman is a semiconductor invention, and its disclosure relates to the electronic aspects of the system. There is no practical detail about the chemistry or the reagents present in solution. The system is for detection only. There is no suggestion that it might be used for sequence analysis.

Macevicz describes a standard dot-blot assay format, in which a target is immobilized and addressed by labeled probes, typically 7 to 11 bases in length, in solution. This is remote from applicant's technique where the probes are immobilized and addressed by a target in solution.

Wood is apparently cited, merely to show the use of tetramethylammonium chloride in hybridization assays. Applicant acknowledges that Wood does disclose this feature.

Applicant's claim 40 is unobvious from Hafeman in several respects:

- The claim is directed to apparatus for analyzing an undetermined sequence or undetermined sequence variant of a polynucleotide. In other words, applicant's apparatus is designed to generate sequence information. In contrast, Hafeman barely mentions nucleic acid hybridizations, and is, in any event, concerned with methods of detection.

- Claim 40 recites an array of different oligonucleotides with defined sequences. Hafeman contains no corresponding disclosure, either of using oligonucleotides as

opposed to polynucleotides or nucleic acids, or as to providing probes of defined sequence.

- Claim 40 recites an arrangement such that application to the array of a polynucleotide sequence to be analyzed under hybridization conditions allows oligonucleotides which have complements in the polynucleotide to be distinguished from those which do not. Skill and care are required to make an array of immobilized oligonucleotides which permit hybridization events to be distinguished, and applicant's specification contains considerable teaching to this end. There is absolutely no corresponding disclosure or suggestion in Hafeman, who indeed barely mentions nucleic acids at all.

Claim 41 recites that the oligonucleotides of each cell of the array are attached to the surface of a support through a terminal nucleotide. There is no corresponding disclosure or suggestion in Hafeman.

Claim 42 recites that the oligonucleotides of each cell have been synthesized *in situ* and are attached to the surface of a support through a covalent linkage. There is no corresponding disclosure or suggestion in Hafeman.

Claim 43 recites that the oligonucleotide of each cell has a length of 8 to 20 nucleotides. There is no corresponding disclosure or suggestion in Hafeman.

The features which unobviously distinguish method claim 53 over the disclosure of Hafeman are essentially the same as

those which distinguish the corresponding apparatus claim 40 discussed above.

Claims 49 and 52 are allowable by virtue of being dependent on allowable claims 41, 42 and 43.

Claim 50 recites that the oligonucleotides immobilized in the array have a length of from 8 to 20 nucleotides. In contrast, Macevicz uses a mixture of oligonucleotides in solution, where each has a length of 7 to 11 nucleotides. Macevicz does not teach or suggest providing immobilized oligonucleotides as probes.

Claim 59 is patentable as dependent upon claim 53.

Old claims 19, 22, 23 and 38 were rejected under 35 USC 103 as unpatentable over Fodor. The new claims corresponding to these old claims are 44, 47, 48 and 46.

The Fodor reference was published on February 5, 1991. It discloses work that was inspired by the invention described in the present application. Fodor is not citable against any of claims 44 to 48, all of which are fairly based on the British priority application of May 3, 1988 or at least on the U.S. application filed May 2, 1989 (see the above discussion on priority dates of claims).

Lastly, Fodor contains no disclosure that is not also present in Serial No. 07/573,317 filed May 2, 1989. (If the Examiner disagrees, he is invited to point to the relevant disclosure in Fodor.) If this assertion is correct, then it must

surely follow that either claims 44 to 48 are entitled to a May 2, 1989 date, or that Fodor is fatally deficient as a reference against the claims.

Disclosure Objections.

The Official Action has objected to certain passages in the body of the specification. In reply:

A new page 1 is filed herewith, in which the words on lines 33 to 37 are clearly presented.

On page 13 line 20, the figures "$3x10^{-12}$ μmol" and "2g" have been changed to read "$3x10^{-12}$ mmol" and "2 mg". The units concerning loading on CPG are in error: the load on a patch 1 x 10 x 10 microns, should be $3x10^{-12}$ mmol and the amount of human DNA to which this is equivalent should be 2 mg. The molecular weight of human genomic DNA is ca. $6x10^{12}$ for haploid nuclei. Thus, the amount of oligonucleotide bound to CPG is equivalent to:

$$3 \times 10^{-12} \times 6 \times 10^{12} \approx 2 \text{ mg}.$$

On page 15, line 12 the phrase has been corrected to read "G + C content".

The typographical error in claim 34 has been corrected in the new claims submitted herewith.

Lastly, applicant notes with appreciation that old claims 20 and 36, equivalent to new claims 45 and 58, are deemed allowable over the cited prior art.

No further issues remaining, allowance of this application is respectfully requested.

If the Examiner has any comments or proposals for expediting prosecution, he is invited to contact the undersigned at the telephone or facsimile number below.

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